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Stem Cell Culture

Neuronal Cell Culture

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Cellular Hypoxia Discovery

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Transforming Cell Culture with Low Oxygen:

The Critical Case for Physioxia

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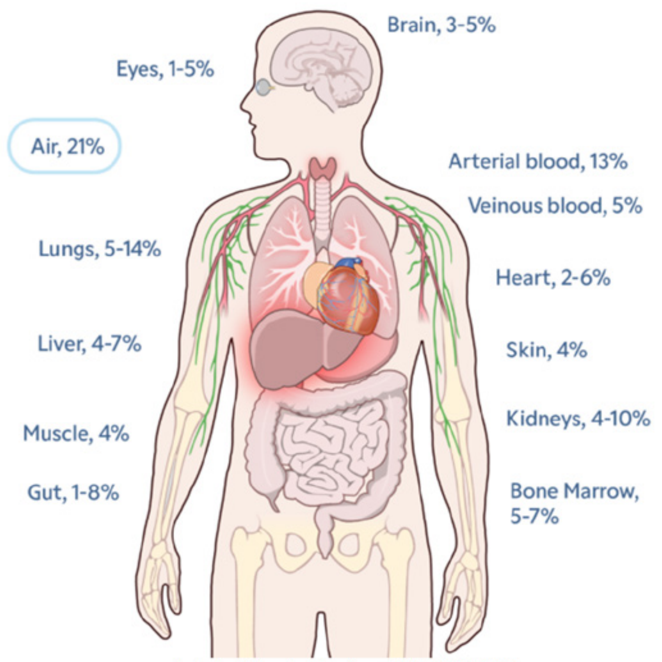
Advancing Scientific Discovery with Physioxic Cell Culture

Physioxia is critically important for stem cell culture, neuronal cell culture, and the generation of ACTs.

In most conventional labs, cells are cultured under normoxic conditions, meaning the oxygen level is at 21%, matching atmospheric oxygen. However, this does not reflect the natural environment of many human cell types, which typically experience physiological oxygen levels (physioxia) of just 1 to 8 %, as shown in Figure 1. Consequently, cells cultured under normoxia can experience stress and altered metabolism, due to increased reactive oxygen species that can cause mitochondrial damage, ultimately leading to inaccurate experimental data. In contrast, culturing cells under physioxia is known to enhance viability, function, and differentiation, ensuring more reliable results. This eBook looks at how researchers' understanding of hypoxia has developed over the years, before delving into the critical importance of physioxia for stem cell culture, neuronal cell culture, and the generation of adoptive cell therapies (ACTs).



Adapted illustration from NIH BIOART Source.

Figure 1. Typical physioxic conditions in the human body.

Key Advances in Cellular Hypoxia Discovery

Researchers' understanding of cellular hypoxia dates back to the late 19th century, when Louis Pasteur reported that yeast has an increased capacity to ferment under depleted oxygen. This observation was followed by a landmark study showing that tumor cells favor aerobic glycolysis to meet their energy needs, even when oxygen is abundant—a phenomenon that became known as the Warburg effect. Other key advances include the discovery of hypoxia-inducible factor 1 (HIF-1) and the subsequent elucidation of oxygen-sensing mechanisms in human cells, which saw William Kaelin Jr., Sir Peter Ratcliffe, and Gregg Semenza receive the 2019 Nobel Prize in Physiology or Medicine. More recently, a HIF-2 inhibitor (belzutifan) has been approved by the US Food and Drug Administration (FDA) for treating renal cell carcinomas.

For a complete timeline of cellular hypoxia discoveries see the *Key Advances in Cellular Hypoxia Discovery infographic* timeline on page **18.**

The Impact of Physioxia on Gene Expression

Culturing cells under physioxic conditions can lead to significant changes in gene expression. These are mainly driven by HIFs—transcription factors which respond to low oxygen levels. To date, three HIFs have been discovered (HIF-1, HIF-2, and HIF-3), each consisting of two subunits, α and β. In all cases, the β subunit is consistently expressed in the nucleus, independent of oxygen levels, while the α subunit exhibits differential responses to normoxia and hypoxia. HIF-1α, the original HIF isoform identified by Semenza *et al.,* is the most widely studied α subunit. It is expressed in most human cells, whereas HIF-2α and HIF-3α are expressed only in certain tissues and cell types.

Under normoxic conditions, HIF-1α is hydroxylated on specific proline residues by prolyl hydroxylase domain-containing enzymes (PHDs), a process requiring oxygen as a cofactor. This marks

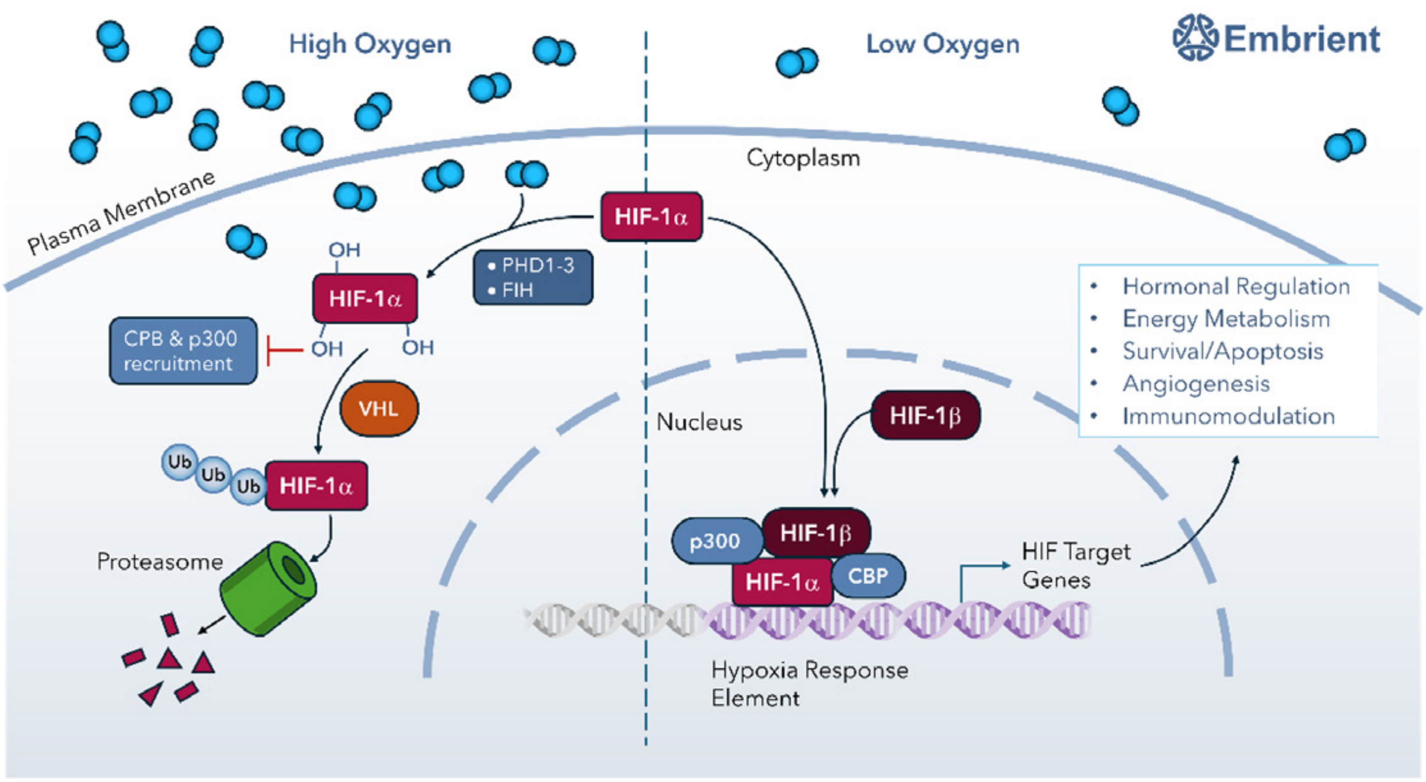


Figure 2. HIF-1α under conditions of normoxia and hypoxia.

it for recognition and ubiquitination by the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex, leading to its proteasomal degradation. Under hypoxic/physioxic conditions, the activity of PHDs is inhibited, resulting in the stabilization and accumulation of HIF-1α, which can then translocate to the nucleus. Here, it dimerizes with HIF-1β, forming an active transcriptional complex that binds to hypoxia-responsive elements (HREs) in the promoters of various target genes, activating or repressing their expression. This process is shown in Figure 2.

Physioxic Cell Culture for Research, Drug Discovery, and Therapeutics

Adopting physiologic conditions for cell culture can have far-reaching implications for research, drug discovery, and therapeutic applications. Not only does physioxia improve cell health, but it also allows for designing more accurate *in vitro* models that better reflect *in vivo* conditions. This is particularly important when working with sensitive cell types like stem cells, immune cells, and neurons, which thrive in lower oxygen environments, as well as for developing a better understanding of the tumor microenvironment (TME).

In **Chapter 2**, we explore the importance of physioxia in stem cell culture. Within this setting, physioxic conditions can promote the expression of genes associated with pluripotency and self-renewal, thereby maintaining stem cells in an undifferentiated state. They can also direct stem cell differentiation toward specific lineages, depending on the context. Key molecular pathways involved in hypoxia are discussed, followed by strategies for optimizing stem cell culture conditions.

Chapter 3 investigates the role of physioxia in neuronal cell culture, with a focus on practical considerations for replicating physioxic conditions *in vitro*. These include the use of oxygen control systems, such as Embrient's Modular Incubator Chamber, to create hypoxic environments and the potential need for adjustments to the composition of culture media. In addition, key applications are highlighted, including disease modeling for conditions like ischemia, Alzheimer's disease, and Parkinson's disease, where oxygen dynamics can influence cell behavior.

In **Chapter 4**, physioxia and adoptive cell therapies take center stage. Specifically, the impact of physiological oxygen levels on cell metabolism, differentiation, survival, and function are covered, all of which are essential for developing successful treatments. This chapter also comments on how severe hypoxia in the tumor microenvironment can limit ACT efficacy, as well as suggests strategies for improving T cell infiltration and function. Lastly, manufacturing and scalability challenges are addressed, along with future directions.

About Embrient

Embrient specializes in developing simple-to-use, low-cost products to support physioxic cell culture. The company's Modular Incubator Chamber facilitated the Nobel Prize winning work of William Kaelin Jr., Sir Peter Ratcliffe, and Gregg Semenza and has been widely cited in the literature for applications spanning basic research through to the development of advanced therapeutics.

Stem Cell Culture Requires Low Oxygen

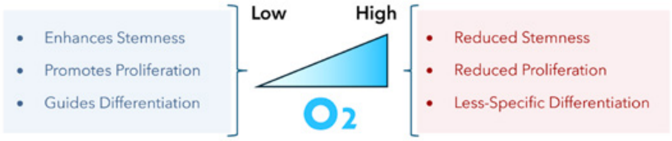
By refining our understanding and control of hypoxic conditions, researchers can improve the efficiency and reliability of stem cell-based therapies.

Hypoxia-Enabled Stem Cell Culture

Stem cell research has revolutionized regenerative medicine and tissue engineering. A critical factor in advancing stem cell culture is the optimization of the microenvironment, particularly oxygen levels. Hypoxia, or reduced oxygen availability that mimics the physiological state (physioxia), has emerged as a pivotal condition for maintaining stem cell potency and promoting specific differentiation pathways. This chapter explores the importance of hypoxia in stem cell culture, the molecular pathways involved, and its implications for optimizing stem cell applications.

Importance of Hypoxia in Stem Cell Culture

In vivo, stem cells often reside in niches characterized by low oxygen levels (3–13%), such as the bone marrow or the embryonic inner cell mass. These hypoxic/physioxic conditions play a crucial role in regulating stem cell self-renewal, proliferation, and differentiation. Mimicking this natural environment *in vitro* has been shown to optimize stem cell culture, while culture at atmospheric oxygen levels produces suboptimal and unwanted results.



1. Enhances Stemness:

- Hypoxic conditions maintain the pluripotency of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) by stabilizing key transcription factors such as OCT4, SOX2, and NANOG.

2. Promotes Proliferation:

- Hypoxia enhances the proliferation rate of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs).

3. Guides Differentiation:

- Low oxygen tension can direct stem cell differentiation toward specific lineages, such as chondrocytes or cardiomyocytes, depending on the context.

In addition, low oxygen also affects motility and adhesion of stem cells, influencing the expression of adhesion molecules and proteins required for cytoskeletal reorganization.

Molecular Pathways Involved in Hypoxia versus Hyperoxia Responses

Cellular responses to extremes in oxygen levels are mediated by a complex network of molecular pathways, the most notable being the Hypoxia-Inducible Factor (HIF) signaling pathway and the Nuclear factor erythroid 2-Related Factor 2 (NRF2) pathway.

1. HIF Stabilization and Activation:

- Under normoxic conditions, HIF-α subunits are hydroxylated by prolyl hydroxylase domain (PHD) enzymes, marking them for degradation via the ubiquitin-proteasome pathway.
- In hypoxic conditions, the lack of oxygen inhibits PHD activity, allowing HIF-1α to accumulate and translocate to the nucleus, where it dimerizes with HIF-1β.
- The HIF complex binds to hypoxia-responsive elements (HREs) in hundreds of target genes, upregulating pathways involved in glycolysis, angiogenesis, and cell survival.

2. Metabolic Reprogramming:

- Hypoxia shifts cellular metabolism from oxidative phosphorylation to glycolysis, reducing reactive oxygen species (ROS) production and protecting cells from oxidative damage.
- This metabolic reprogramming supports the maintenance of pluripotency and enhances cell survival under low oxygen conditions.

3. Epigenetic Regulation:

- Hypoxia influences the expression of chromatin-modifying enzymes, such as histone demethylases, which further regulate genes associated with stemness and differentiation.

4. Wnt/β-Catenin Pathway Interaction:

- Hypoxia has been shown to enhance the activity of the Wnt/β-catenin pathway, a critical regulator of stem cell fate.

5. NRF2 Activation:

- The transcription factor NRF2, encoded by the NFE2L2 gene, is a crucial master regulator for maintaining cellular redox homeostasis in response to oxidative stress, which is often a result of excess O_2.
- Under normal conditions, NRF2 is bound by two molecules of KEAP1, an adapter protein for E3 ubiquitin ligases, resulting in consistent degradation by the 26S proteasome.
- Under oxidative stress conditions, ROS or electrophiles interact with or modify KEAP1, resulting in conformational changes in the KEAP1 protein that disrupt the NRF2-KEAP1 complex. Free NRF2 translocates to the nucleus, initiating the binding of the NRF2–sMAF complex to antioxidant response elements (AREs), resulting in the transactivation of target genes.
- NRF2 activation triggers the expression of hundreds of genes involved in various cellular processes, including antioxidant and xenobiotic responses, cell proliferation and survival, and metabolism, among others.

HIF-1 and NRF2 share a complex network of interactions both positively and negatively influencing the other's expression and activity depending on the cellular context. Both are integral for stem cell function and survival.

Implications for Optimizing Stem Cell Culture

Leveraging hypoxia in stem cell culture requires careful control of oxygen levels and an understanding of its effects on cell behavior. Here are some strategies and considerations for optimizing culture conditions:

1. Oxygen Tension Control:

- Specialized hypoxia chambers or incubators can maintain oxygen levels between 1–5%, mimicking physiological hypoxia. Embrient's Modular Incubator Chamber (MIC-101) is ideal for low or custom oxygen cell culture. It is simple-to-use and easy to clean, while its sealed environment minimizes consumption of expensive gas mixes.
- Real-time oxygen monitoring ensures consistency and reproducibility.

2. Supplementation with Hypoxia-Mimetic Agents:

- Compounds like cobalt chloride ($CoCl_2$) or dimethyloxalylglycine (DMOG) can stabilize HIF-α, simulating hypoxic effects without altering atmospheric oxygen levels.

3. Lineage-Specific Applications:

- Hypoxia can be tailored to promote specific differentiation pathways for targeted therapeutic applications. Examples include:

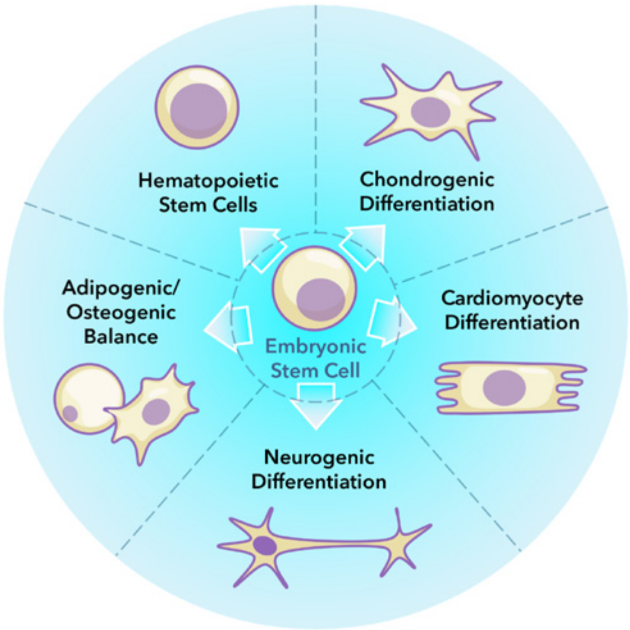
» **Chondrogenic Differentiation:** Mesenchymal stem cells (MSCs) cultured under hypoxia show enhanced chondrogenic differentiation, making it a promising strategy for cartilage repair and regeneration. Hypoxia upregulates SOX9, a key transcription factor in chondrogenesis, and promotes the deposition of extracellular matrix components like collagen type II.

» **Cardiomyogenic Differentiation:** Hypoxic conditions favor the differentiation of stem cells into cardiomyocytes, a critical application for cardiac tissue engineering and the treatment of myocardial infarction. This is mediated through HIF-dependent pathways that enhance the expression of cardiac-specific markers such as NKX2.5 and troponin T.

» **Neurogenic Differentiation:** Neural stem cells (NSCs) benefit from hypoxia by exhibiting increased proliferation and differentiation into neurons and glial cells. Hypoxia-induced factors, including vascular endothelial growth factor (VEGF), play a role in enhancing neurogenesis.

» **Adipogenic and Osteogenic Balance:** Hypoxia can regulate the balance between adipogenic and osteogenic differentiation of MSCs. While low oxygen levels inhibit adipogenesis, they enhance osteogenic differentiation by modulating pathways like BMP and RUNX2.

» **Hematopoietic Stem Cells:** Hypoxic culture conditions maintain the quiescence and self-renewal of hematopoietic stem cells (HSCs), critical for bone marrow transplants and hematological therapies.



Challenges and Future Directions

Despite its benefits, hypoxia-enabled culture presents challenges, including the risk of variability in oxygen delivery and the need for standardization across labs. Future research should focus on:

- Developing more robust hypoxia-mimetic systems.
- Investigating the interplay between hypoxia and other niche factors, such as extracellular matrix and mechanical cues.
- Exploring long-term effects of hypoxia on genomic stability and therapeutic potential.
- Overcoming the common practice of culturing cells at normoxic conditions.

Conclusion

Hypoxia plays a fundamental role in optimizing stem cell culture by mimicking the natural microenvironment, activating critical molecular pathways, and directing cell fate decisions. By refining our understanding and control of hypoxic conditions, researchers can improve the efficiency and reliability of stem cell-based therapies, paving the way for advances in regenerative medicine and beyond.

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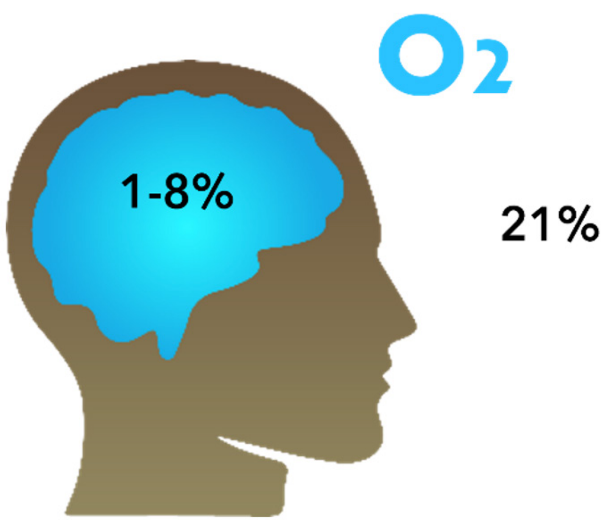
Physiological Oxygen for Neuronal Cell Culture

The choice of oxygen levels in neuronal cell culture has profound implications for cell behavior, experimental outcomes, and translational relevance.

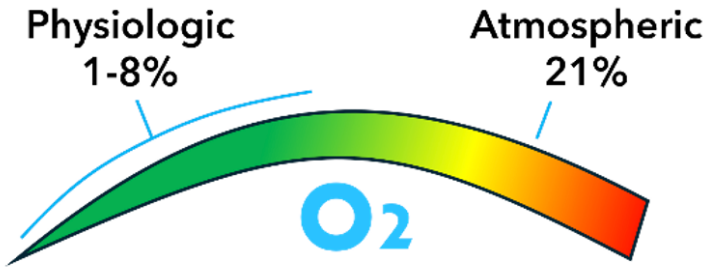
Introduction

The oxygen concentration in the cellular environment is a critical determinant of cell physiology and function. In the context of neuronal cell culture, the choice of oxygen levels can significantly influence the behavior, differentiation, and viability of neurons. Atmospheric oxygen levels (~21%) are commonly used in standard *in vitro* culture conditions; however, they do not reflect the physiological oxygen levels found in tissues. In the brain, oxygen levels range between 1% and 8%, a state referred to as hypoxia or, more accurately, physiological oxygen tension. This technical note explores the differences in neuronal cell behavior under low oxygen (physiological) conditions compared to atmospheric oxygen conditions and provides insights into optimizing neuronal cell culture systems.



Oxygen Levels in the Brain

The brain is highly metabolically active and consumes approximately 20% of the body's oxygen despite constituting only 2% of the total body mass. Within the brain, oxygen gradients exist due to variations in blood supply and cellular metabolic demand. While oxygen levels in arterial blood entering the brain may approach 8%, within microenvironments like the hippocampus or the cortical layers, oxygen can drop to as low as 1–3%. These gradients underscore the need to replicate physiological oxygen levels experienced by cells *in vitro* to better model *in vivo* neuronal behavior.



- HIF-1 activation
- Mitochondrial integrity
- Enhanced cell viability
- Improved neuronal differentiation

- Increased oxidative stress
- Damaged mitochondria
- Increased apoptosis
- Impaired neuronal differentiation

Atmospheric Oxygen Versus Physiological Oxygen

Effects on Neuronal Differentiation

Atmospheric oxygen levels can induce oxidative stress in neurons, as they are exposed to higher-than-physiological reactive oxygen species (ROS) levels. ROS can interfere with neuronal differentiation by disrupting signaling pathways such as those mediated by hypoxia-inducible factors (HIFs). Physiological oxygen, by contrast, maintains HIF activity, which is crucial for the regulation of neurogenesis, axonal guidance, and synaptic plasticity. Studies have shown that culturing neural stem cells (NSCs) and progenitor cells in physiological oxygen enhances their differentiation into mature neuronal phenotypes while maintaining a better balance between proliferation and differentiation.

Cellular Metabolism and Energy Production

Neurons are highly dependent on oxidative phosphorylation for ATP production. However, exposure to atmospheric oxygen can alter the metabolic profile of neurons, leading to an increased reliance on glycolysis due to oxidative damage to mitochondria. Physiological oxygen levels mitigate this by reducing mitochondrial ROS production, thus preserving mitochondrial integrity and ensuring efficient ATP production. This is particularly important in long-term cultures where sustained energy production is critical.

Viability and Survival

Neuronal cells are sensitive to oxidative stress, which can lead to apoptosis or necrosis under atmospheric oxygen conditions. In physiological oxygen environments, reduced oxidative stress enhances cell viability and reduces markers of apoptosis. For example, studies have demonstrated that neurons cultured at 3% oxygen exhibit lower levels of caspase activation and DNA fragmentation compared to those cultured at 21% oxygen.

Mechanisms Underlying Oxygen-Mediated Effects

Reactive Oxygen Species (ROS)

ROS play a dual role in neuronal cultures: at physiological levels, they act as signaling molecules regulating cellular processes, while at elevated levels, they cause oxidative damage. Atmospheric oxygen conditions result in excessive ROS generation, leading to lipid peroxidation, protein oxidation, and DNA damage. In contrast, physiological oxygen levels maintain ROS within a range that supports signaling without inducing damage.

Hypoxia-Inducible Factors (HIFs)

HIFs are transcription factors stabilized under low oxygen conditions. They regulate genes involved in angiogenesis, energy metabolism, and cell survival. Atmospheric oxygen suppresses HIF activity, which may lead to reduced adaptability of neurons to metabolic or environmental stress. Physiological oxygen, on the other hand, supports the stabilization of HIFs, promoting adaptive responses essential for neuronal health.

Practical Considerations for Neuronal Cell Culture

Oxygen Control Systems

To replicate physiological oxygen levels, specialized hypoxia chambers or incubators with adjustable oxygen control are required. These systems allow precise regulation of oxygen concentration, ensuring that cells experience conditions akin to their *in vivo* environment. It is crucial to monitor oxygen levels throughout the culture period, as fluctuations can introduce variability in experimental outcomes. Learn more about how Embrient's Modular Incubator Chamber provides a convenient, low-cost solution for controlled hypoxia environments.

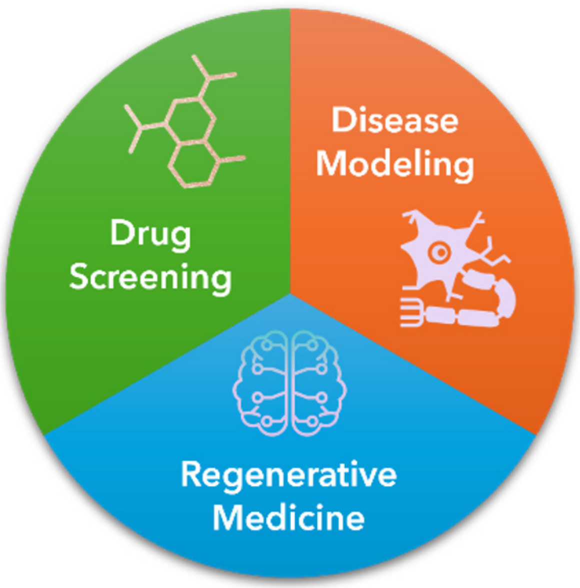
Media and Supplements

Oxygen levels influence nutrient utilization and metabolic pathways. Therefore, the composition of culture media may need adjustment when working under physiological oxygen conditions. For instance, glucose concentrations may be optimized to match the reduced glycolytic flux observed in neurons cultured under low oxygen.

Long-Term Cultures

Long-term neuronal cultures are particularly prone to oxidative stress when maintained in atmospheric oxygen. Physiological oxygen conditions not only improve cell survival but also preserve neuronal morphology and function over extended periods. This is especially relevant for studies involving synaptic plasticity, network formation, or neurodegenerative disease modeling.

Applications and Implications



Disease Modeling

Physiological oxygen is critical for modeling diseases like ischemia, Alzheimer's, and Parkinson's, where oxygen dynamics play a central role. Mimicking *in vivo* oxygen conditions enhances the relevance of these models and may provide new insights into disease mechanisms and therapeutic targets.

Drug Screening

The response of neurons to drugs can differ significantly between atmospheric and physiological oxygen conditions. Culturing neurons under physiological oxygen ensures that drug screening assays more accurately reflect *in vivo* pharmacodynamics and toxicology.

Neuroengineering and Regenerative Medicine

Physiological oxygen enhances the maturation and functionality of neuronal constructs in neuroengineering applications. Similarly, it improves the success rates of neuronal grafts and other regenerative medicine strategies by better preparing cells for *in vivo* transplantation.

Conclusion

The choice of oxygen levels in neuronal cell culture has profound implications for cell behavior, experimental outcomes, and translational relevance. While atmospheric oxygen conditions are convenient, they fail to replicate the *in vivo* microenvironment of neurons. Physiological oxygen levels better support neuronal differentiation, metabolism, and survival, making them indispensable for advanced research applications. By adopting oxygen-controlled culture systems and optimizing protocols, researchers can achieve more accurate and reproducible results, bridging the gap between *in vitro* studies and *in vivo* realities.

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Optimizing Adoptive Cell Therapies with Low Oxygen

Understanding and harnessing the role of oxygen in T cell biology will be essential for developing next-generation adoptive cell therapy strategies.

Oxygen levels play a critical role in the generation and efficacy of adoptive cell therapies (ACT), a form of immunotherapy that involves engineering and infusing immune cells, such as T cells, into patients to combat diseases like cancer and autoimmunity. The significance of oxygen levels in this process can be understood through its impact on cell metabolism, differentiation, survival, and function, all of which are essential for the success of ACT.

1. Cell Metabolism and Energy Production

Oxygen is a key regulator of cellular metabolism, particularly in immune cells like T cells, which are central to adoptive cell therapies. T cells rely on oxidative phosphorylation (OXPHOS) in the mitochondria for energy production in oxygen-rich environments. However, under low oxygen (hypoxic) conditions, such as those found in tumor microenvironments, T cells switch to glycolysis, a less efficient but faster energy-producing pathway. This metabolic shift can influence T cell differentiation and function. For instance, hypoxic conditions often promote the generation of memory T cells, which are long-lived and highly effective in mounting sustained immune responses. Conversely, high oxygen levels may favor the differentiation of effector T cells, which are more short-lived but potent in immediate tumor killing. Understanding and manipulating oxygen levels during the *ex vivo* expansion of T cells can thus optimize their metabolic state for therapeutic efficacy.

2. T Cell Differentiation and Phenotype

Oxygen levels significantly influence T cell differentiation, which is crucial for adoptive cell therapies. Hypoxia-Inducible Factors (HIFs), transcription factors activated under low oxygen conditions, play a pivotal role in shaping T cell phenotypes, acting as master regulators of hundreds of genes. HIF-1α, for example, promotes the differentiation of T cells into Th17 cells, which are associated with inflammation, while suppressing regulatory T cells (Tregs), which can inhibit anti-tumor immunity. For cell therapies, the balance between effector T cells

(e.g., cytotoxic T cells) and Tregs is critical. Hypoxic conditions during T cell expansion may enhance the generation of tumor-specific cytotoxic T cells while minimizing Treg activity, thereby improving therapeutic outcomes.

3. T Cell Survival and Persistence

The survival and persistence of adoptively transferred T cells *in vivo* are key determinants of adoptive cell therapy success. Oxygen levels during the *ex vivo* culture phase can influence the expression of survival factors and resistance to apoptosis. For example, hypoxic conditions have been shown to upregulate anti-apoptotic proteins like Bcl-2, enhancing T cell survival post-infusion. Additionally, hypoxia can promote the generation of stem-like memory T cells (Tscm), which have superior persistence and self-renewal capabilities compared to effector T cells. These long-lived T cells are highly desirable for ACT, as they can provide durable anti-tumor responses.

4. Tumor Microenvironment and T Cell Function

The tumor microenvironment (TME) is often characterized by severe hypoxia, which poses a challenge for the efficacy of ACT. Hypoxic TMEs can impair T cell function by inducing exhaustion, a state of reduced cytotoxicity and cytokine production. Exhausted T cells express inhibitory receptors like PD-1 and CTLA-4, which limit their anti-tumor activity. Preconditioning T cells under hypoxic conditions during *ex vivo* expansion may enhance their adaptability and function in the hypoxic TME. Additionally, strategies to modulate oxygen levels in the TME, such as combining ACT with anti-angiogenic therapies or oxygen delivery systems, could improve T cell infiltration and function.

5. Manufacturing and Scalability

Oxygen levels also impact the scalability and reproducibility of ACT manufacturing. Traditional T cell expansion protocols often use ambient oxygen levels (21%), which may not reflect physiological conditions. However, physiological oxygen levels in tissues range from 1% to 11%, with even lower levels in the TME. Culturing T cells under physiologically relevant oxygen conditions can improve their functionality and reduce oxidative stress, which can damage cells during expansion. Moreover, hypoxia can enhance the yield of certain T cell subsets, such as Tscm, which are desirable for ACT. Optimizing oxygen levels during manufacturing is thus essential for producing high-quality, clinically effective T cell products.

6. Clinical Implications and Future Directions

The manipulation of oxygen levels in ACT has significant clinical implications. For example, hypoxia-conditioned T cells have shown enhanced anti-tumor activity in preclinical models, suggesting that oxygen modulation could improve ACT outcomes in patients. Additionally, combining ACT with therapies that target hypoxia, such as HIF inhibitors or oxygen-carrying nanoparticles, could further enhance efficacy. Future research should focus on defining optimal oxygen conditions for different T cell subsets and developing standardized protocols for oxygen modulation in ACT manufacturing.

Conclusion

Oxygen levels are a critical factor in the generation and success of adoptive cell therapies. By influencing T cell metabolism, differentiation, survival, and function, oxygen modulation can enhance the efficacy of ACT and improve patient outcomes. As the field of immunotherapy advances, understanding and harnessing the role of oxygen in T cell biology will be essential for developing next-generation adoptive cell therapy strategies.

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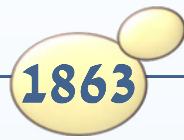
Key Advances in Cellular Hypoxia Discovery

An Infographic Timeline

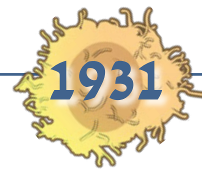
Pasteur Effect



Louis Pasteur observed that oxygen inhibits fermentation in yeast, indirectly introducing the idea of oxygen's effect on cellular metabolism.

Significance: First connection between oxygen levels and cellular metabolic pathways.

Warburg Hypothesis



Otto Warburg identified the metabolic shift in cancer cells, favoring glycolysis even in the presence of oxygen (aerobic glycolysis).

Significance: Laid groundwork for understanding cellular metabolism under altered oxygen conditions.

Hypoxia and Erythropoiesis

Leon Jacobson and colleagues demonstrated hypoxia-induced erythropoiesis, the process of red blood cell production.

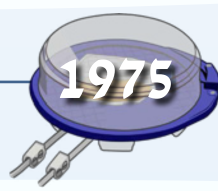
Significance: Highlighted hypoxia as a physiological regulator.

EPO Discovery

Eugene Goldwasser and colleagues purified erythropoietin (EPO), a hormone promoting red blood cell production.

Significance: Identified EPO as a hypoxia-induced hormone, linking cellular signaling to oxygen sensing.

Hypoxia Chamber



Jim Billups and Barry Rothenberg develop the first Modular Incubator Chamber for controlling low oxygen cell culture environments.

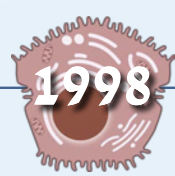
Significance: Enabled low-cost hypoxia tissue culture research.

Discovery of HIF-1

Gregg Semenza and Guang Wang published findings on hypoxia-inducible factor 1 (HIF-1).

Significance: Established HIF-1 as a key regulator of cellular response to hypoxia, controlling gene expression in low-oxygen conditions.

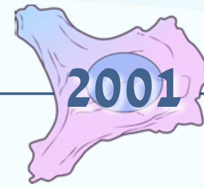
Metabolic Reprogramming



Semenza and colleagues uncovered the role of hypoxia in reprogramming cellular metabolism via HIF-1, promoting glycolysis and lactate production.

Significance: Provided insights into hypoxia's role in homeostasis, cancer and other diseases.

HIF-1 Regulation



William Kaelin Jr. and colleagues characterized oxygen-dependent hydroxylation of HIF-1 and subsequent ubiquitination and proteasomal degradation.

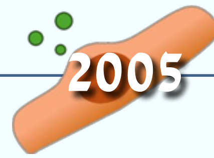
Significance: Clarified the role of oxygen in regulating HIF-1 function.

Mitochondrial Role in Hypoxia

Navdeep Chandel and colleagues revealed that mitochondria play a central role in hypoxic signaling by producing reactive oxygen species (ROS) under low oxygen.

Significance: Connected mitochondrial function with cellular adaptation to hypoxia.

Role of VEGF



Vascular endothelial growth factor (VEGF) was identified as a hypoxia-induced mediator of angiogenesis by Napoleone Ferrara.

Significance: Demonstrated the role of hypoxia in vascular remodeling and its relevance to diseases like cancer.

Nobel Prize

William Kaelin Jr., Sir Peter Ratcliffe, and Gregg Semenza received the Nobel Prize for their groundbreaking discoveries related to oxygen-sensing mechanisms. All three laureates acknowledged using the Modular Incubator Chamber to create hypoxic environments in their research.

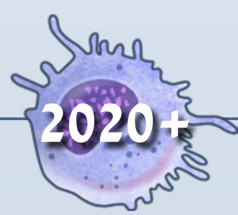
Significance: Recognized decades of work revealing how cells adapt to oxygen availability, cementing the field's importance.

HIF-2 Inhibitor Approved

FDA approved belzutifan, a HIF-2 inhibitor, for treating cancers like renal cell carcinoma.

Significance: Marked the transition of hypoxia research into therapeutic applications.

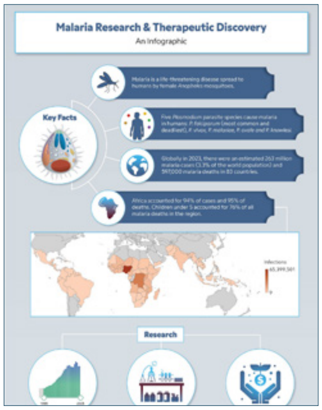
Recent Advances



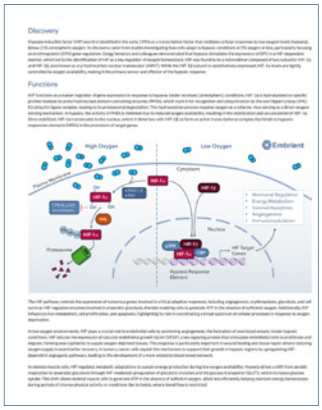
Many researchers continue advancing our understanding of hypoxic mechanisms, particularly in immune regulation, stem cell biology, infectious disease, and tissue regeneration, leading to improved cell culture modeling, optimized therapeutic cell cultures, and targeting hypoxia in disease.

Significance: Results have established the wide-reaching influence of hypoxia on cellular processes, with implications for therapies in ischemia, cancer, and beyond.

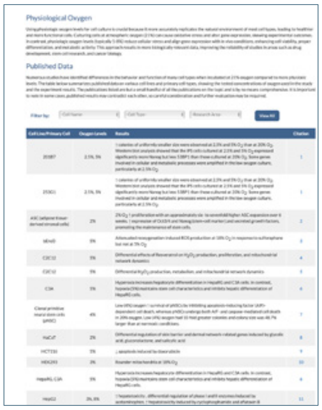
Resources



Malaria Research and Therapeutic Discovery Infographic

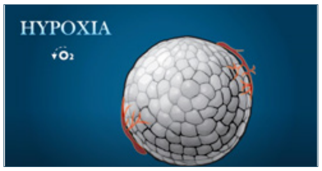


HIF-1α Discovery, Function, and the 2019 Nobel Prize Technical Note

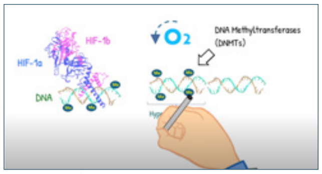


Summary of Published Physioxic Cell Culture Conditions

Cellular Transcriptome and Proteome Dynamics Under Hypoxia Technical Note



Hypoxia and the Tumor Microenvironment Video



Epigenetics and Hypoxia Video

IMAGINE

A complete solution for maximizing cell culture reproducibility at an exceptionally affordable price

The Pioneer Bundle, *featuring:* the Gas Mixing System

The Gas Mixing System (GMS-5003) is a state-of-the-art flow-through oxygen (O_2) sensor designed to be used in-line with the Dual Flow Meter (DFM-3002) to precisely measure the percentage of oxygen for flushing and filling the Modular Incubator Chamber (MIC-101).

The Bundle includes: Modular Incubator Chamber, a Dual Flow Meter, the Gas Mixing System, tubing clamps (10 pack), air filters (6 pack), and tubing (3 meters).

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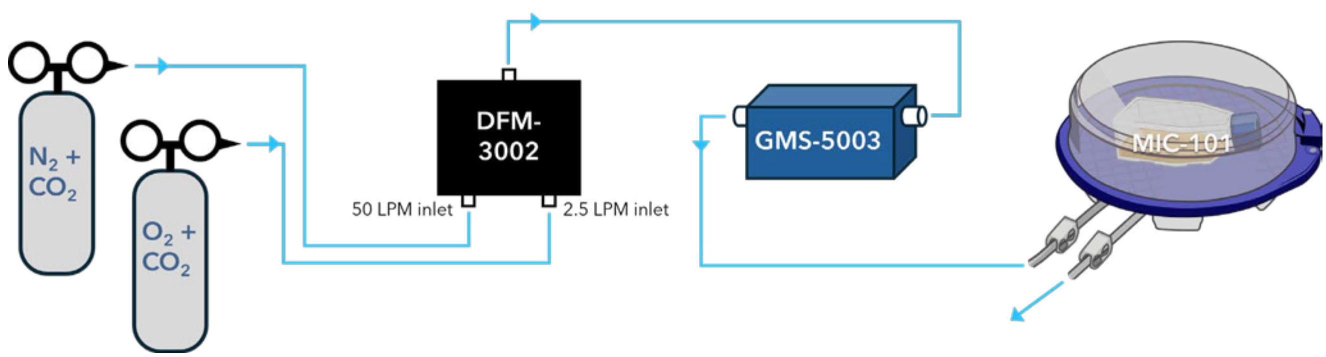
The setup:



Embrient Inc

Formerly known as billups-rothenberg inc

Tel: (858) 535-0545
Toll-Free: (877) 755-3309
Email: info@embrient.com